

# Fusarium oxysporum f. sp. vasinfectum 5.8s rRNA gene and adjacent ITS1 and ITS2 regions

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## Abstract

*Fusarium oxysporum*, Schlecht ex Fr. is a phytopathogenic fungus causing wilting or yellows disease on a variety of plant species throughout the world. It is categorized in *formae speciales* according to pathotypic variation and physiological character (Messiaen and Cassini, 1981 *Fusarium:- Diseases, Biology and Taxonomy* pp.427-445). The *F. oxysporum forma specialis vasinfectum* (Atk.) Snyder and Hansen is pathogenic on cotton (*Gossypium* spp.) on which it causes severe damage to susceptible races. We report here the DNA sequence of the 5.8S rRNA gene and flanking intergenic transcribed spacers of *F. oxysporum forma specialis vasinfectum*. DNA was isolated from mycelial cultures from three virulent isolates collected from single cotton plants from geographically distant sites in Bié, Cuanza Norte and Cuanza Sul regions of Angola (Ragazzi, 1992 J. Pl. Disease Protect. 99:499-504).

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## ***Fusarium oxysporum f. sp. vasinfectum* 5.8s rRNA gene and adjacent ITS1 and ITS2 regions**

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*Fusarium oxysporum*, Schlecht ex Fr. is a phytopathogenic fungus causing wilting or yellows disease on a variety of plant species throughout the world. It is categorized in *formae speciales* according to pathotypic variation and physiological character (Messiaen and Cassini, 1981 *Fusarium:- Diseases, Biology and Taxonomy* pp.427-445). The *F. oxysporum forma specialis vasinfectum* (Atk.) Snyder and Hansen is pathogenic on cotton (*Gossypium* spp.) on which it causes severe damage to susceptible races. We report here the DNA sequence of the 5.8S rRNA gene and flanking intergenic transcribed spacers of *F. oxysporum forma specialis vasinfectum*. DNA was isolated from mycelial cultures from three virulent isolates collected from single cotton plants from geographically distant sites in Bié, Cuanza Norte and Cuanza Bul regions of Angola (Ragazzi, 1992 *J. Pl. Disease Protect.* 99:499-504).

Ribosomal DNA (rDNA) repeat units contain highly conserved DNA sequences which have been used to detect phylogenetic relationships between species, as well as more variable DNA sequence regions which have been used to detect genetic variation between related fungal species and strains (White et al, 1990 *PCR Protocols: A Guide to Methods and Applications.* pp.315-322. Academic Press; O'Donnell, 1992 *Curr. Genet.* 22:213-220). The intergenic transcribed sequence (ITS) comprises the transcribed region flanking the 5.8S gene and is located between the 3' of the 18S gene and the 5' of the following 28S gene. Amplification of this region by the polymerase chain reaction (PCR) using DNA primers specific for conserved 18S and 28S elements has been used to produce characteristic DNA fragments from yeasts and from filamentous fungi. Genetic relationships between fungal strains have been deduced from sequence variation found in the ITS1 and ITS2 intergenic transcribed spacer regions, or from characteristic RFLP maps resulting from differential restriction of the region (O'Donnell 1992 *Curr. Genet.* 22:213-220; Kasuga et al, 1993 *Curr. Genet.* 24:343-346). Notably, sequence variation in the ITS regions of fungi was often not associated with a restriction site and was not detected by RFLP mapping.

### **Results**

Cuanza Bul and Cuanza Norte isolates of *F. oxysporum f.sp. vasinfectum* had identical ITS1 and ITS2 regions of 151 bp and 152 bp. respectively. The Cuanza Bul isolate varied from Bi, and Cuanza Norte isolates by a single T deletion in the ITS1 region at nucleotide 37 (Figure 1). Restriction polymorphism was reported to be absent from the rRNA gene repeat of many *forma speciales* of *F. oxysporum* (Kistler et al, 1987 *Phytopath.* 77:1289-1293). The variation reported here within *forma speciales vasinfectum* was detectable by DNA sequencing and does not occur within any known restriction site. This suggests that nucleotide variation undetected by restriction analysis may occur within the rRNA gene repeat other races of *F. oxysporum*. Alignment using the FASTA program (Pearson and Lipman, 1988 *Proc. Natl. Acad. Sci. USA* 85:2444-2448) showed the 5.8S gene of the *F. sambucinum* to be 100% homologous to the *F. oxysporum* gene. Examination of the sequence the ITS regions of races of *F. sambucinum*



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251      260      270      280      290      300
Foxyv TTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGA
      |||
Fsam  TTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGA

ITS2 -->
301      310      320      330      340      350
Foxyv GCGTCATTTCAACCCTCAAGCACAGCTTGGTGTGGGA-CTCGCGTTAAT
      |||
Fsam  GCGTCATTTCAACCCTCAAGCCCAGCTTGGTGTGGGAGCTGTCGT---C

351      360      370      380      390      400
Foxyv TCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGT
      | | ||| |||| |
Fsam  TGACACTCCCCAAATACATTGGCGGTCACGTCGAGCTTCCATAGCGTAGT

401      410      420      430      440      450
Foxyv AGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCA
      | | | | |||
Fsam  AATTTACACATCGTTACTGGTAATCGTCGCGGCCACG-CGTTAAA-CCCA

451      460      470
Foxyv ACTTCTGAATG
      |||
Fsam  ACTTCTGAATG

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**Figure 1.** Nucleotide sequence of the antisense strand of the 5.8S rRNA gene of the *F. oxysporum f.sp. vasinfectum* (Foxyv) from Angola. Representative sequences are shown in comparison with *F. sambucinum* (Fsam) (EMBL: X65482) (O'Donnell 1992 Curr. Genet. 22: 213-220). Bold symbols indicate the 5.8S rRNA gene. The nucleotide 37 (***bold italic***) in the ITS1 region of Cuanza Bul and Cuanza Norte isolates is absent in Bié isolate. EMBL Accession Nos. for the sequences reported in this article are: X78258, X78259, X78260.